AMENDMENT UNDER 37 C.F.R. 1.116

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hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, [and] wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*; and

(b) freezing the cell suspension to yield a frozen cell suspension.

Please add the following claims:

- (New) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- (New) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- 55. (New) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the composition does not comprise



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dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

- (New) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- (New) A method for preserving cells comprising: freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.
- 58. (New) A method for preserving cells comprising:
- (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum;

Light Contact

Docket No. 600.451US1 XD #476205

CLEAN VERSION OF PENDING CLAIMS

OMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD

LYMPHOCYTES

Applicant: Allison Hubel Serial No.: 09/458,862

- 1
- 1. (Four times amended) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, which agent is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, a biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- 2. The cryopreservation medium of claim 1 wherein the cells are peripheral blood lymphocytes.
- 3. The cryopreservation medium of claim 1 that comprises arabinogalactan.
- 4. The cryopreservation medium of claim 1 further comprising a cryoprotective agent that penetrates the cell membrane.
- 5. The cryopreservation medium of claim 4 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 6. The cryopreservation medium of claim 1 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.

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- 7. The cryopreservation medium of claim 1 which does not comprise protein.
- 8. The cryopreservation medium of claim 1 which is infusible.
- 11. The cryopreservation medium of claim 1 wherein the cells are human cells.
- 12. The cryopreservation medium of claim 1 wherein the cells are non-human vertebrate cells.
- 14. A composition suitable for administration to a human, comprising a suspension of cells in a cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, and a cryoprotective agent that penetrates the cell membrane, wherein arabinogalactan, or a biological or functional equivalent thereof, is present in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum.
- 16. The composition of claim 14 wherein the cells are peripheral blood lymphocytes.
- 17. The composition of claim 14 wherein at least one of the cryoprotective agents is arabinogalactan.
- 19. The composition of claim 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

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20. The composition of claim 14 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.

- 21. The composition of claim 14 which does not comprise protein.
- 22. The composition of claim 14 which is infusible.
- 24. The composition of claim 14 wherein the cells are human cells.
- 26. (Twice amended) A method for preserving cells comprising:
- (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*; and
 - (b) freezing the cell suspension to yield a frozen cell suspension.
- 27. The method of claim 26 further comprising thawing the frozen cell suspension under conditions that maintain cell viability.
- 28. The method of claim 26 wherein the cells are human cells.

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30. The method of claim 26 wherein the cells are peripheral blood lymphocytes

31. A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum.

- 32. A frozen hematopoietic cell-containing composition made according to the method of claim 26.
- 33. The cryopreservation medium of claim 5 wherein the cryoprotective agent that penetrates the cell membrane is glycerol.
- 34. The cryopreservation medium of claim 33 wherein the concentration of glycerol is about 1% to about 3%.
- 35. The cryopreservation medium of claim 1 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.
- 36. The composition of claim 14 or 31 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.
- 37. A cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination

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thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the balanced electrolyte solution is selected from the group consisting of lactated Ringer's solution, PlasmaLyte-ATM, Normosol-RTM, Veen-DTM, Polysal®, and Hank's balanced salt solution.

- 38. The cryopreservation medium of claim 37 wherein the lymphocytes are peripheral blood lymphocytes.
- 39. The cryopreservation medium of claim 37 wherein the agent is arabinogalactan.
- 40. The cryopreservation medium of claim 37 further comprising a cryoprotective agent that penetrates the cell membrane.
- 41. The cryopreservation medium of claim 40 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 42. The cryopreservation medium of claim 37 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.
- 43. The cryopreservation medium of claim 37 which does not comprise protein.
- 44. The cryopreservation medium of claim 37 which is infusible.

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47. The cryopreservation medium of claim 37 wherein the cells are human cells.

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- 48. The cryopreservation medium of claim 37 wherein the cells are non-human vertebrate cells.
- 49. The method of claim 26 wherein the medium comprises arabinogalactan.
- 50. The method of claim 26 further comprising a cryoprotective agent that penetrates the cell membrane.
- 51. The method of claim 50 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.
- 52. The method of claim 26 wherein the lymphocytes which are modified ex vivo are activated lymphocytes or genetically modified lymphocytes.
- 53. (New) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells. lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.

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54. (New) A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.

- 55. (New) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- (New) A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified *ex vivo*.
- 57. (New) A method for preserving cells comprising: freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan

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in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are modified ex vivo.

- 58. (New) A method for preserving cells comprising:
- (a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are modified ex vivo, or a combination thereof, and wherein the medium does not comprise dimethylsulfoxide or serum; and
- (b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.